REMARKS

Claims 4-24 remain pending in this application. Claims 4, 5, 8 and 10 are currently amended only as to matters of grammar and form and to further clarify the intended subject matter. No new matter has been added.

Applicants submit that this Amendment After Final Rejection should only require a cursory review because the claim amendments presented herein do not add any new features and/or do not significantly alter the scope of the claims. Consequently, the claim amendments should not require any further search by the Examiner. Accordingly, entry of the present Amendment, as an earnest attempt to advance prosecution is requested under 37 C.F.R. §1.116.

CLAIM REJECTION - 35 USC § 102

At page 3, the Office Action maintains the rejection of claims 4-6, 10-14, 16-18 and 22 under 35 U.S.C. § 102(b) as being anticipated by HINDS et al. (Enhanced gene replacement in mycobacteria, Microbiology, 1999, Vol. 145: 591-527). Applicants respectfully traverse the rejection.

The present application relates to the finding that a replication competent DNA molecule treated with a mutagenic substance that blocks its replication, when transformed into a prokaryotic or eukaryotic cell, undergoes much higher levels of

homologous recombination than an untreated DNA molecule. Thus, the treated DNA molecule is a far superior agent for transforming prokaryotic or eukaryotic cells by targeted homologous recombination. This method overcomes some of the limitations associated with previous methods of transforming a prokaryotic or eukaryotic cell by targeted homologous recombination.

As described in the present specification, the increased level of homologous recombination is likely due to the generation of highly "recombinogenic" ends resulting from partial replication of the treated DNA molecule in the target cell. These highly recombinogenic ends remain active as the replication of the DNA molecule cannot become complete due to the treatment of the DNA molecule with the mutagenic/replication blocking substance.

As such, claim 4 is directed to a method for *in vitro* insertion of a nucleic acid of interest within a predetermined target nucleotide sequence present in a chromosome. The method includes in part:

a) providing a DNA vector that is replication competent in the target cell comprising the nucleic acid of interest and contacting the DNA vector with a mutagenic agent blocking intracellular DNA replication of the DNA vector to produce a modified DNA vector;

b) transfecting the target cells with the modified DNA vector obtained under conditions wherein replication of the modified DNA vector commences and insertion of the nucleic acid of interest within the predetermined target nucleotide sequence occurs; and

c) selecting cells where the nucleic acid of interest has been integrated into the predetermined target nucleotide sequence.

HINDS fails to teach or suggest such a method. HINDS describes a method for homologous recombination (HR) in *Mycobacterium* that uses plasmid vectors treated with UV radiation, or so called "suicide vectors." In contrast to the present claimed method, these suicide vectors are not replication competent and do not replicate in the target cells (mycobacteria).

The Office Action appears to misinterpret the methodology of HINDS. In particular, the recombination assays using the pRAM4 shuttle vector are based upon an intraplasmid recombination event and do not lead to the introduction of a sequence at a target in the host cell genome. Indeed, what the authors in HINDS are looking for when using the pRAM4 vector is the loss or retention of a kanamycin resistance cassette positioned between two parts of a hygromycin resistance cassette. This means that the non-recombined pRAM4 vector will give rise to

kanamycin resistant/hygromycin sensitive colonies and the recombined pRAM4 vector will give rise to kanamycin sensitive/hygromycin resistant colonies.

Again, the method of present claim 4 features the insertion of a nucleic acid of interest initially included in a DNA vector within a predetermined target nucleotide sequence in the genome of a target cell. In distinction, the HINDS recombination assays in *Mycobacterium* using pRAM4 were performed "To identify experimental conditions for the application of gene replacement experiments that favoured HR, a plasmid-based recombination assay was developed which would generate quantitative results. The recombination assay vector, pRAM4, was constructed based upon restoration of a functional *hyg* gene by an HR event between two overlapping fragments of *hyg* gene flanking a functional *kan* gene in a shuttle plasmid (Fig. la, b). Intraplasmid HR between the duplicated DNA would restore a complete and functional *hyg* gene, deleting the *kan* cassette in the process" (emphasis added), (see, page 521, column 2, paragraph 2). Thus, HINDS discloses an intraplasmid HR assay, i.e., an assay of HR events within the same plasmid, using the pRAM4 shuttle vector. No insertion of any sequence contained within the pRAM4 shuttle vector into the host cell genome occurs or is tested using this assay.

Also, the Office Action's contention that the pRAM4 vector is treated with a mutagen that prevents replication of this vector is not correct. In fact, it is the presence or absence of the replicated progeny of the original or recombined pRAM4 shuttle vector in the later generations of the target cell that allows the authors to measure the effects of the various mutagens on intra-pRAM4 vector recombination. HINDS fails to teach or suggest the use of anything other than a suicide vector for the introduction of a DNA sequence by HR into a target cell genome. HINDS also does not teach that a replication competent vector would be used for such a purpose.

For at least these reasons, HINDS fails to teach or suggest, and does not anticipate, the methods of claims 4-6, 10-14, 16-18 and 22. Accordingly, Applicants request reconsideration and withdrawal of the rejection.

CLAIM REJECTION - 35 USC § 103

At page 5, the Office Action rejects claims 4-21 and 23-24 under 35 U.S.C. § 103(a) as being unpatentable over HOEIJMAKERS et al. (US 2003/0124605) in view of HINDS. Applicants respectfully traverse the rejection.

First, as detailed in the above remarks, HINDS fails to teach or suggest any method having the combination of features as recited in the present claims.

Second, the Office Action appears to have misinterpreted HOEIJMAKERS. HOEIJMAKERS relates to methods to monitor the levels of HR23 protein binding partners in cells following exposure to a DNA modifying agent (i.e., a mutagen). The HR23 protein binding partners investigated include XPC, MAG, CREB, and p53. HR23 and its binding partners are one of the *in vivo* mechanisms involved in repairing DNA lesions in the host cell genome.

The cells used in this method were engineered so as to have altered expression of HR23A and/or HR23B. For HR23A, this was achieved by targeted gene disruption in accordance with the scheme shown in Figure 1. The strategy outlined in Figure 1 and detailed at page 9, paragraphs 108-115, is a known homologous recombination gene alteration strategy. This strategy involves the generation of a mHR23A targeting vector which has two portions of homology to exons II and exons VII/VIII of the HR23A gene and flanking a neomycin resistance cassette (see, Figure 1 and paragraph [0108]).

This non-replication competent targeting vector (it is a bacterial plasmid and the target is a mammalian cell) is then electroporated into the target cells. Following culturing and expansion of the initial target population, G418 resistant clones are selected based upon the presence of the neomycin resistance cassette as a stable integrant. This is verified by way of two

restriction enzyme digests of genomic DNA from the selected clones to verify that the clones contained the neomycin resistance cassette in the correct position in the genome disrupting the HR23A gene (see, Figure 1 and paragraph [0110]).

Using these transformed cells, transgenic mice and immortalized cell lines were created which had a homozygous $HR23A^{-/-}$ genotype. The $HR23A^{-/-}$ mice were interbred with existing $HR23B^{-/-}$ mice (Ng et al., Mol. Cell Biol. 22, 1233-1245, 2002) to produce homozygous double mutant $HR23A^{-/-}$ $B^{-/-}$ mice (see, paragraph [0112]).

For HR23B, a full length cDNA of this gene was inserted into the pSLM vector and then transfected into DKO MEF (Double-Knockout Mouse embryo fibroblasts) cells from the mice bred in paragraph [0112], together with a separate vector comprising a full length cDNA of the hXPC-GFP fusion protein. The resulting transformed cells were then selected for stable transformants of these transgenes based upon the presence of the puromycin resistance cassette present in the vectors (see, paragraphs [0129] and [0130]). The vectors used in these experiments are not replication competent, they randomly insert themselves into the target cell genome as per the normal transfection protocol.

Therefore, HOEIJMAKERS details two methods of transforming a target cell population:

1) The method used to knock-out the HR23A gene, by inserting via a homologous recombination event a neomycin resistance gene which is inserted in place of several exons of the HR23A gene removing its function; and

2) The method used to insert (knock-in) the HR23B and hXPC-GFP transgenes using transfection vectors and the selection of stable transformants again on the basis of the insertion of the neomycin resistance gene into the host cell genome.

Only one of these methods uses homologous recombination as the means of transforming the host cell (Method 1) and in this transformation method it is noted that the "mHR23A Targeting Vector" is not capable of replicating in the target cell (because it is a bacterial plasmid and the target cell is mammalian). Also, at no point prior to or following the introduction of this targeting vector into the target cell was it exposed to any mutagen such as UV or a chemical mutagen. Such exposure only occurred after the stable clone resulting from the HR event had been selected for and isolated.

HOEIJMAKERS prepared their panel of HR23A/B knock-out / knock-in mouse cell lines so that the effects of mutagens on these cell lines could be studied. HOEIJMAKERS is not concerned with, and fails to teach or suggest anything about, methods of transforming a cell so as to introduce a nucleic acid sequence of interest other than using standard known techniques such as by

way transfecting a cell and selecting for transformants (method 2) or introducing a non-replication competent DNA targeting vector with homologous portions to those of a target genomic locus, which following a rare homologous recombination event leads to the insertion of a selectable marker and some change in the locus which is then selected for based upon the insertion of a selectable resistance cassette. The subsequent treatment of these HR23A and/or HR23B knock-out/knock-in cell lines (what the Office Action refers to as "cells stably expressing hXPC-GFP/hHR23B were rinsed with PBS, exposed to UV... and subsequently cultured at 37°C for various time periods...") refers to the exposure of stably transformed cell lines which comprise the hXPC-GFP/hHR23B transgenes stably chromosomally integrated, with the specified mutagen so that the effects of this in the engineered genetic background can be assessed.

This exposure of the cells to a mutagen is therefore not so that these already stably integrated transgenes can become integrated as the Office Action suggests. Thus, HOEIJMAKERS teaches the same "standard" strategy for performing a genetic transformation event using homologous recombination, that is, the homologous recombination vector is a suicide vector (it cannot replicate in the target cell) so that transformants which result from homologous recombination can be selected without a massive background of the selection cassette being present as a

non-genomically integrated plasmid (or other genetic structure) in the target cell.

For all of these reasons, the combination of HOEIJMAKERS and HINDS fails to teach or suggest, and fails to render obvious, the methods of present claims 4-21 and 23-24. Accordingly, Applicants request reconsideration and withdrawal of the rejection.

CLAIM REJECTION – 35 USC § 112

At page 9, the Office Action rejects claims 4 under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully traverse the rejection.

Currently amended claim 4 further clarifies the intended subject matter and addresses the issues noted in the Office Action. Accordingly, Applicants request reconsideration and withdrawal of the rejection.

CONCLUSION

Entry of the above amendments is earnestly solicited. Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

The Commissioner is hereby authorized in this, concurrent, and future submissions, to charge any deficiency or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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